

a.) Amendment to the Claims

1. (Currently Amended) A method for producing a neural crest cell or a neural tube cell, comprising:

~~pre-culturing inducing differentiation of an embryonic stem cell into a neural stem cell or nerve cell *in vitro*, which comprises culturing the embryonic stem cell under non-aggregation conditions, wherein said culturing is carried out in the absence of retinoic acid, acid and in the presence of a stroma cell without forming embryoid body for a time period from 1 day to 14 days so that differentiation of embryonic stem cell into a nervous system cell before determining of dorso-vental axis; and then~~

culturing the pre-cultured embryonic stem cell in vitro in the absence of retinoic acid and in the presence of both BMP-4 and a stroma cell without forming embryoid body.

Claims 2-14 (Cancelled).

15. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 80 or 81, which further comprises culturing under serum-free culture conditions.

Claims 16-17 (Cancelled)

18. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 80 or 81, wherein the stroma cell is a stroma cell whose proliferation potency is deleted by a physicochemical treatment.

19. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 80 or 81, wherein the stroma cell is a stroma cell whose proliferative potency is deleted by an antitumor agent, ~~irradiation~~ irradiation or pathologic tissue fixative.

20. (Previously Presented) The method according to claim 18, wherein the physicochemical treatment is an antitumor agent selected from the group consisting of mitomycin C, 5-fluorouracil, adriamycin and methotrexate.

21. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 80 or 81, wherein the stroma cell is a stroma cell whose proliferative potency is deleted by a microwave fixation, a rapid freeze-substitution fixation, a glutaraldehyde fixation, a p-formaldehyde fixation, a formalin fixation, an acetone fixation, a Van fixation, a periodic acid fixation, a methanol fixation ~~and~~ or an osmic acid fixation.

22. (Previously Presented) The method according to claim 23, wherein the stroma cell is recognized by a monoclonal antibody produced by hybridoma FERM BP-7573.

23. (Currently Amended) The method according to any one of claims 1, ~~14, 15 and 18-21~~ 80 or 81, wherein the stroma cell is selected from the group consisting of: a fetal primary culture fibroblast; an SIHM mouse-derived STO cell; a mouse fetus-derived NIH/3T3 cell; an M-CSF deficient mouse calvaria-derived OP9 cell; a mouse calvaria-derived MC3T3-G2/PA6 cell; an embryonic stem cell-derived stroma cell; and a bone marrow mesenchymal stem cell-derived stroma cell.

24. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 80 or 81, wherein the embryonic stem cell is selected from the group consisting of:

- (a) an embryonic stem cell established by culturing an early embryo before implantation;
- (b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and
- (c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering.

Claims 25-55 (Cancelled)

56. (Currently Amended) A method for evaluating a substance for activity in regulating differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell, which comprises:

carrying out the method according to ~~either of claims 1 or~~ claim 24 both (i) in the presence of a substance to be tested and (ii) in the absence of the substance to be tested; and

comparing differentiation of the embryonic stem cell into an ectodermal cell obtained in the presence of the substance to be tested with that in the absence of the substance to be tested.

57. (Currently Amended) A method for screening a substance for activity in regulating differentiation of an embryonic stem cell into an ectodermal cell, which comprises:

carrying out the method according to ~~either of claims 1 or~~ claim 24 both (i) in the presence of a substance to be tested and (ii) in the absence of the substance to be tested; and

comparing differentiation of the embryonic stem cell into an ectodermal cell obtained in the presence of a substance to be tested with that in the absence of the substance to be tested.

Claims 58-71 (Cancelled).

72. (Currently Amended) The method according to ~~either of claims 1 or claim 24~~, wherein the stroma cell is ~~PA6~~ MC3T3-G2/PA6, OP9 or NIH3T3.

Claim 73 (Cancelled)

74. (Previously Presented) The method according to claim 23, wherein the embryonic stem cell is selected from the group consisting of:

- (a) an embryonic stem cell established by culturing an early embryo before implantation;
- (b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and
- (c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering.

75. (Previously Presented) The method according to claim 74, wherein the stroma cell is recognized by a monoclonal antibody produced by hybridoma FERM BP-7573.

76. (Previously Presented) The method according to claim 56, wherein the stroma cell is selected from the group consisting of: a fetal primary culture fibroblast; an SIHM mouse-derived STO cell; a mouse fetus-derived NIH/3T3 cell; an M-CSF deficient mouse calvaria-derived OP9 cell; a mouse calvaria-derived MC3T3-G2/PA6 cell; an embryonic stem cell-derived stroma cell; and a bone marrow mesenchymal stem cell-derived stroma cell.

77. (Previously Presented) The method according to claim 76, wherein the embryonic stem cell is selected from the group consisting of:

(a) an embryonic stem cell established by culturing an early embryo before implantation;

(b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and

(c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering.

78. (Previously Presented) The method according to claim 57, wherein the stroma cell is selected from the group consisting of: a fetal primary culture fibroblast; an SIHM mouse-derived STO cell; a mouse fetus-derived NIH/3T3 cell; an M-CSF deficient mouse calvaria-derived OP9 cell; a mouse calvaria-derived MC3T3-G2/PA6 cell; an embryonic stem cell-derived stroma cell; and a bone marrow mesenchymal stem cell-derived stroma cell.

79. (Previously Presented) The method according to claim 78, wherein the embryonic stem cell is selected from the group consisting of:

- (a) an embryonic stem cell established by culturing an early embryo before implantation;
- (b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and
- (c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering.

80. (New) A method for producing a dopaminergic neuron, an acetylcholinergic neuron, a γ -aminobutyrate neuron or a serotonergic neuron, comprising:

pre-culturing an embryonic stem cell *in vitro* in the absence of retinoic acid and in the presence of a stroma cell without forming embryoid body for a time period from 1 day to 14 days so that differentiation of embryonic stem cell into a nervous system cell before determining of dorso-vental axis; and then

culturing the pre-cultured embryonic stem cell *in vitro* in the absence of both retinoic acid and BMP-4 and in the presence of a stroma cell without forming embryoid body.

81. (New) A method for producing a neural stem cell which is stained by an anti-nestin antibody comprising

culturing an embryonic stem cell *in vitro* in the absence of both retinoic acid and BMP-4 and in the presence of a stroma cell without forming embryoid body for a time period from 1 day to 14 days.

82. (New) The production method according to claim 1, wherein the cell is said neural crest cell.

83. (New) The production method according to claim 1, wherein the cell is said neural tube cell.

84. (New) The production method according to claim 80, wherein the neuron is said dopaminergic neuron.

85. (New) The production method according to claim 80, wherein the neuron is said acetylcholinergic neuron.

86. (New) The production method according to claim 80, wherein the neuron is said γ -aminobutyrategic neuron.

87. (New) The production method according to claim 80, wherein the neuron is said serotonergic neuron.